I INTERFERENCE OF FLUORIDE WITH COLORIMETRIC MEASUREMENT OF PHOSPHORUS

II NITROGEN NUTRITION STUDIES WITH CORN

by

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INTRODUCTION

Excellent correlation between crop response to application of phosphatic fertilizer and extraction of available phosphorus by the Bray and Kurtz (2) method has been illustrated recently by Smith, et al (3). However, it has been shown by Kurtz (1) that the presence of the fluoride ion affects the color development in the colorimetric method commonly used for phosphorus determination. Kurtz also showed that the addition of H_3BO_3 to a solution containing fluoride eliminated, to a certain extent, the interference with the color development.

The Kansas state soil testing laboratory has adopted the Bray and Kurtz (2) method for determining available phosphorus in soils. To overcome the repression of the color development caused by the fluoride ion the $(NH_4)_2MoO_4$--HCl solution is saturated with H_3BO_3.

Because the presence of fluoride in the extracting solution does affect the color development, and because H_3BO_3 is used to overcome this difficulty, an experiment was designed to determine the effect of different concentrations of fluoride on the color development and to determine to what extent the H_3BO_3 in the $(NH_4)_2MoO_4$--HCl solution may be depended upon to overcome this interference. The experiment was conducted with phosphorus standards in water, 0.025 N HCl, and 0.1 N HCl.

EXPERIMENTAL METHODS

In order to determine the effect of fluoride on color development, standards containing 1, 2, 3, and 4 ppm phosphorus were used. Color was developed by using $(NH_4)_2MoO_4$--HCl solution with amino-naphthol-sulfonic acid as the reducing agent. Intensity of color was determined by the use of an Evelyn photoelectric colorimeter. Determinations were first made in the absence of fluoride in order to give a basis for comparison; then determinations were made with different amounts of NH_4F added to the solution.

Secondly, in order to determine the effect of H_3BO_3 in reducing the interference of color development caused by the fluoride ion, the experiment was repeated using $(NH_4)_2MoO_4$--HCl solution with H_3BO_3 added. Progressive rates of NH_4F (0.03 N, 0.04 N, 0.05 N, and 0.06 N) were used to determine the concentration at which interference first occurs.

Thirdly, both preceding steps in the experiment were repeated with phosphorus standards in 0.025 N HCl and 0.1 N HCl instead of water.

In all cases where H_3BO_3 was used it was added with the $(NH_4)_2MoO_4$--HCl solution. This solution was prepared saturated with respect to H_3BO_3.

REAGENTS

Standard Phosphorus Solution

Stock solution containing 50 ppm phosphorus was prepared by dissolving 0.2195 gm of dry KH_2PO_4 in water and diluting to one liter. Working standards were prepared by quantitative dilution of the stock solution.

Standard Fluoride Solution

Approximately 2 N solution was prepared by dissolving 74.08 gms of NH_4F in water and diluting to one liter. This solution was standardized against $Th(NO_3)_4$ using $Zr(NO_3)_2$, alizarin red mixture as an indicator.

Standard Hydrocholoric Acid

Approximately 1 N solution was prepared by diluting 83 mls concentrated HCl to one liter. This solution was standardized against a base.

Amino-Naphthol-Sulfonic Acid

Two and one-half gms 1-amino-2-naphthol-4-sulfonic acid, 5.0 gms Na_2SO_3, and 146.25 gms $Na_2S_2O_5$ were thoroughly mixed and ground to a fine powder. Eight gms of the powder mixture were dissolved in 50 mls of warm distilled water. It was filtered before using.

Ammonium Molybdate-Hydrochloric Solution

One-hundred gms of $(NH_4)_2MoO_4$ were dissolved in 850 mls of distilled water. It was filtered and cooled. A second solution consisting of 1700 mls of concentrated HCl mixed with 160 mls of water was made. The first solution was slowly added to the second solution and constant stirring was employed. One-hundred ten gms of reagent grade H_3BO_3 was added to the above mixture for the $(NH_4)_2MoO_4$--HCl-H_3BO_3 solution.

PROCEDURE

From the 50 ppm phosphorus stock solution 10, 20, 30, and 40 ppm phosphorus solutions were prepared by diluting 20, 40, 60, and 80 mls respectively of the 50 ppm phosphorus stock solution to 100 mls. Five mls of each (10, 20, 30, and 40 ppm phosphorus) were diluted to 50 mls with distilled water in order to give 1, 2, 3, and 4 ppm phosphorus solutions. A blank solution consisted of 50 mls of distilled water.

The above solutions were transferred to 125 mls Erlenmeyer flasks. Two mls $(NH_4)_2MoO_4$--HCl solution and 2 mls of amino-naphthol-sulfonic acid were added. This was mixed and allowed to stand for 15 minutes. At the end of 15 minutes, readings on the Evelyn photoelectric colorimeter were made using a wavelength of 660 mu.

Each of the succeeding determinations was made as above except when diluting the 10, 20, 30, and 40 ppm phosphorus solutions to give 1, 2, 3, and 4 ppm phosphorus solutions to give 1, 2, 3, and 4 ppm phosphorus certain concentrations of NH_4F and / or HCl were used in place of distilled water. The solutions used to dilute the standards were prepared from standard NH_4F and HCl solutions. For each determination a blank solution containing everything except the phosphorus standards was prepared.

DISCUSSION AND RESULTS

Table 1 presents the effect of fluoride on color development. Standard phosphorus solutions were in water. The $(NH_4)_2MoO_4$--HCl solution was used without H_3BO_3. The addition of 0.03 N NH_4F repressed the color considerably. The greatest recovery with the standards in 0.03 N NH_4F was 61 per cent for

concentration of only 1 ppm phosphorus and the least was 48 per cent for concentration of 4 ppm phosphorus. As the concentration of phosphorus increased per cent recovery decreased. In 0.06 N NH_4F no color developed.

Table 1. Phosphorus standards in water, $(NH_4)_2MoO_4$--HCl solution without H_3BO_3.

Phosphorus added to solution	Normality with respect to NH_4F	Phosphorus[1] found in solution	Per cent recovery
1 ppm	0.00	1.00 ppm	100.0
2 ppm	0.00	2.00 ppm	100.0
3 ppm	0.00	3.00 ppm	100.0
4 ppm	0.00	4.00 ppm	100.0
1 ppm	0.03	0.61 ppm	61.0
2 ppm	0.03	1.16 ppm	58.0
3 ppm	0.03	1.62 ppm	54.0
4 ppm	0.03	1.95 ppm	48.8
1 ppm	0.06	0.00 ppm	0.0
2 ppm	0.06	0.00 ppm	0.0
3 ppm	0.06	0.00 ppm	0.0
4 ppm	0.06	0.00 ppm	0.0

1 Mean of two duplicates.

When standards were placed in 0.03 N NH_4F plus 0.025 N HCl a greater decrease occurred than in 0.03 N NH_4F (Table 2). Only 50 per cent recovery occurred with 1 ppm phosphorus. This was 11 per cent decrease compared to 0.03 N NH_4F without the 0.025 N HCl. The least difference occurred with the 4 ppm phosphorus standard, which gave only 7.6 per cent greater decrease than when the standards were in 0.03 N NH_4F. Apparently, the presence of acid activated greater repression of color by fluoride. As was the case with standards in water, color failed to develop in a 0.06 N NH_4F solution.

The presence of 0.03 N NH_4F in 0.1 NHCl greatly repressed color development. Recovery was only 16 per cent in the 3 and 4 ppm phosphorus

standards. This again indicated that fluoride is activated by acid. Apparently the stronger the acid, the greater the activation and, therefore, the greater the color repression. As was true in the two previous trials, color failed to develop in the presence of 0.06 N NH_4F.

Table 2. Phosphorus standards in 0.025 N HCl, $(NH_4)_2MoO_4$--HCl solution without H_3BO_3.

Phosphorus added to solution	Normality with respect to NH_4F	Phosphorus[1] found in solution	Per cent recovery
1 ppm	0.00	1.00 ppm	100.0
2 ppm	0.00	2.00 ppm	100.0
3 ppm	0.00	3.00 ppm	100.0
4 ppm	0.00	4.00 ppm	100.0
1 ppm	0.03	0.50 ppm	50.0
2 ppm	0.03	0.94 ppm	47.0
3 ppm	0.03	1.20 ppm	40.0
4 ppm	0.03	1.65 ppm	41.2
1 ppm	0.06	0.00 ppm	0.0
2 ppm	0.06	0.00 ppm	0.0
3 ppm	0.06	0.00 ppm	0.0
4 ppm	0.06	0.00 ppm	0.0

1 Mean of two duplicates.

Table 3. Phosphorus standards in 0.1 N HCl, $(NH_4)_2MoO_4$--HCl solution without H_3BO_3.

Phosphorus added to solution	Normality with respect to NH_4F	Phosphorus[1] found in solution	Per cent recovery
1 ppm	0.00	1.00 ppm	100.0
2 ppm	0.00	2.00 ppm	100.0
3 ppm	0.00	3.00 ppm	100.0
4 ppm	0.00	4.00 ppm	100.0
1 ppm	0.03	0.19 ppm	19.0
2 ppm	0.03	0.35 ppm	17.5
3 ppm	0.03	0.48 ppm	16.0
4 ppm	0.03	0.64 ppm	16.0
1 ppm	0.06	0.00 ppm	0.0
2 ppm	0.06	0.00 ppm	0.0
3 ppm	0.06	0.00 ppm	0.0
4 ppm	0.06	0.00 ppm	0.0

1 Mean of two duplicates.

Table 4 shows that addition of H_3BO_3 will eliminate interference caused by certain concentrations of NH_4F. Three-hundredths normal NH_4F caused no repression of color development. Four-hundredths normal NH_4F caused no appreciable repression of color development. The first appreciable repression of the color occurred with concentration of 0.05 N NH_4F. One ppm phosphorus in 0.05 N NH_4F was fully recovered. However, recovery dropped to 97 per

Table 4. Phosphorus standards in water, $(NH_4)_2MoO_4$--HCl solution with H_3BO_3.

Phosphorus added to solution	Normality with respect to NH_4F	Phosphorus[1] found in solution	Per cent recovery
1 ppm	0.00	1.00 ppm	100.0
2 ppm	0.00	2.00 ppm	100.0
3 ppm	0.00	3.00 ppm	100.0
4 ppm	0.00	4.00 ppm	100.0
1 ppm	0.03	1.01 ppm	101.0
2 ppm	0.03	2.02 ppm	101.0
3 ppm	0.03	3.00 ppm	100.0
4 ppm	0.03	4.00 ppm	100.0
1 ppm	0.04	1.01 ppm	101.0
2 ppm	0.04	1.97 ppm	98.5
3 ppm	0.04	3.00 ppm	100.0
4 ppm	0.04	3.97 ppm	99.2
1 ppm	0.05	1.00 ppm	100.0
2 ppm	0.05	1.94 ppm	97.0
3 ppm	0.05	2.92 ppm	97.2
4 ppm	0.05	3.87 ppm	96.8
1 ppm	0.06	0.92 ppm	92.0
2 ppm	0.06	1.75 ppm	87.5
3 ppm	0.06	2.62 ppm	87.3
4 ppm	0.06	3.30 ppm	82.5
1 ppm	0.12	0.00 ppm	0.0
2 ppm	0.12	0.00 ppm	0.0
3 ppm	0.12	0.00 ppm	0.0
4 ppm	0.12	0.00 ppm	0.0

1 Mean of two duplicates.

cent for 2 ppm phosphorus. Little variation occurred between the 2, 3, and 4 ppm phosphorus standards. Interference increased with 0.06 N NH_4F. At this concentration of NH_4F it was noticed that the per cent recovery for higher concentrations of phosphorus was less. All concentrations of phosphorus placed in 0.12 N NH_4F failed to produce color.

Table 5. Phosphorus standards in 0.025 N HCl, $(NH_4)_2MoO_4$--HCl solution with H_3BO_3.

Phosphorus added to solution	Normality with respect to NH_4F	Phosphorus[1] found in solution	Per cent recovery
1 ppm	0.00	1.00 ppm	100.0
2 ppm	0.00	2.00 ppm	100.0
3 ppm	0.00	3.00 ppm	100.0
4 ppm	0.00	4.00 ppm	100.0
1 ppm	0.03	1.00 ppm	100.0
2 ppm	0.03	2.00 ppm	100.0
3 ppm	0.03	3.03 ppm	101.0
4 ppm	0.03	4.00 ppm	100.0
1 ppm	0.04	0.99 ppm	99.0
2 ppm	0.04	1.98 ppm	99.0
3 ppm	0.04	2.98 ppm	99.3
4 ppm	0.04	3.92 ppm	98.0
1 ppm	0.05	0.94 ppm	94.0
2 ppm	0.05	1.86 ppm	93.0
3 ppm	0.05	2.75 ppm	91.7
4 ppm	0.05	3.51 ppm	87.8
1 ppm	0.06	0.64 ppm	64.0
2 ppm	0.06	0.97 ppm	48.5
3 ppm	0.06	1.45 ppm	48.3
4 ppm	0.06	1.94 ppm	48.0
1 ppm	0.12	0.00 ppm	0.0
2 ppm	0.12	0.00 ppm	0.0
3 ppm	0.12	0.00 ppm	0.0
4 ppm	0.12	0.00 ppm	0.0

1 Mean of two duplicates.

Table 5 shows the effect of H_3BO_3 in overcoming the interference caused by NH_4F with standards in 0.025 N HCl. As was the case with phosphorus standards in water, no interference resulted with standards in 0.03 N NH_4F plus 0.025 N HCl. Slight repression resulted with standards in 0.04 N NH_4F plus 0.025 N HCl. Phosphorus which was not recovered amounted to about one per cent in most cases. Repression with standards in 0.05 N NH_4F plus 0.025 N HCl and 0.06 N NH_4F plus 0.025 N HCl was considerably greater than repression for corresponding amounts of NH_4F in water. Phosphorus standards in 0.12 N NH_4F failed to produce color.

Table 6. Phosphorus standards in 0.1 N HCl, $(NH_4)_2MoO_4$--HCl solution with H_3BO_3.

Phosphorus added to solution	Normality with respect to NH_4F	Phosphorus[1] found in solution	Per cent recovery
1 ppm	0.00	1.00 ppm	100.0
2 ppm	0.00	2.00 ppm	100.0
3 ppm	0.00	3.00 ppm	100.0
4 ppm	0.00	4.00 ppm	100.0
1 ppm	0.03	1.00 ppm	100.0
2 ppm	0.03	1.95 ppm	97.5
3 ppm	0.03	2.86 ppm	95.3
4 ppm	0.03	3.65 ppm	91.2
1 ppm	0.04	0.80 ppm	80.0
2 ppm	0.04	1.50 ppm	75.0
3 ppm	0.04	2.15 ppm	71.6
4 ppm	0.04	2.70 ppm	67.5
1 ppm	0.05	0.58 ppm	58.0
2 ppm	0.05	1.09 ppm	54.5
3 ppm	0.05	1.57 ppm	52.3
4 ppm	0.05	2.01 ppm	50.2
1 ppm	0.06	0.43 ppm	43.0
2 ppm	0.06	0.75 ppm	37.5
3 ppm	0.06	1.12 ppm	37.5
4 ppm	0.06	1.43 ppm	35.7
1 ppm	0.12	0.00 ppm	0.0
2 ppm	0.12	0.00 ppm	0.0
3 ppm	0.12	0.00 ppm	0.0
4 ppm	0.12	0.00 ppm	0.0

1 Mean of two duplicates.

When different normalities of NH_4F were used with 0.1 N HCl greater repression occurred than when NH_4F was used in water or in 0.025 N HCl. This is clearly shown in Table 6. Some repression occurred with phosphorus standards in 0.03 N NH_4F plus 0.1 N HCl. No repression occurred until 0.04 N NH_4F was present with phosphorus standards in 0.025 N HCl, and no repression occurred until 0.05 N NH_4F was present with phosphorus standards in water. This again showed that the more acid the solution, the greater was repression due to NH_4F.

Per cent recovery progressively decreased with the phosphorus standards in 0.04, 0.05, and 0.06 N NH_4F plus 0.1 N HCl. No color developed in the presence of 0.12 N NH_4F.

SUMMARY

The experiment was designed to determine the effect of different concentrations of fluoride on color development, and to determine to what extent the H_3BO_3 added to $(NH_4)_2MoO_4$--HCl solution may be depended upon to overcome this interference.

The following conclusions were made from the experimental data:

1. Ammonium fluoride represses color development even when present in small concentrations (0.03 N).

2. Ammonium fluoride represses color development more as acidity increases.

3. Color repression is greater for greater concentrations of phosphorus.

4. Boric acid used with $(NH_4)MoO_4$--HCl solution is effective within the following limitations:

a. up to 0.05 N NH_4F in water.

b. up to 0.04 N NH_4F in 0.025 N HCl

c. it was not effective as low as 0.03 N NH_4F in 0.1 N HCl.

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PART II: NITROGEN NUTRITION STUDIES WITH CORN

INTRODUCTION

In recent years interest has developed in use of leaf analyses as diagnostic procedures and for the evaluation of fertility experiments with field crops. Previously, plant analyses had been used to establish many principles of plant nutrition.

The investigation reported in this thesis was designed to study the nitrogen nutrition of corn by use of leaf analysis. Two locations were used in this study, one an irrigated field, and the other a non-irrigated field. Both locations were in northeastern Kansas.

Rates of nitrogen ranging from 20 to 160 pounds of nitrogen per acre were used. Starter fertilizers were incorporated in the irrigated experiment.

In order to study the nitrogen nutrition of corn, leaf samples were taken at various stages of the growing season and analysed in the laboratory. The samples were analyzed for N at both locations, and for P, K, Ca, Mg, and Na at the location which received starter fertilizer.

From the proper interpretation of this study a better understanding of corn nutrition should be gained. This should aid in better fertilizer practices.

REVIEW OF LITERATURE

Use of leaf analyses as a diagnostic procedure is not entirely new. It has been studied for many years in connection with fruit trees. In very recent years it has been installed as a testing service by Michigan State College (Kenworthy, 7). In recent years many attempts have been made to adapt its use to row crops.

Previous investigation with plant analyses for row crops may be divided into two general types:

1. Plant tissue testing by use of rapid tests.
2. Laboratory analyses of plant tissue.

Scarseth (10) utilized the first method in conjunction with corn fertilizer experiments. He used it to study nutrient uptake in an old fertility experiment, and also for the study of fertilizer placement experiments.

Drake (5), also utilizing rapid tissue testing, reported a fair degree of correlation between plant tissue test, fertilizer applied, and yield on Alabama, Georgia, and Mississippi soils. He concluded that, in general, nitrogen was the major factor limiting corn yields.

Leaf analyses have been used not only to study nutrient uptake, but also to predict fertilizer needs for corn. Tyner (12) proposed critical lower limits for content of major fertilizer nutrients in the sixth leaf of the corn plant at tasseling time. These limits were 2.90 per cent nitrogen, .295 per cent phosphorus, and 1.30 per cent potassium.

Much of the previous investigation has been with corn leaves sampled at or near tasseling time. Certain investigators, Tyner (12) and Viets, et al (13), have studied leaf samples taken at various stages of growth. Knauss

(8) studied the nutrient status of corn leaves selected at two stages of growth under Kansas conditions. However, no investigation has been conducted in Kansas in order to evaluate earlier samplings. For this reason leaf samples were gathered at stages of growth earlier than tasseling as well as at tasseling time and after tasseling.

Certain relationships have been reported between various nutrients in the corn leaves. Bennett (3) reported increases in phosphorus content with additions of nitrogen. Knauss (8) reported magnesium-potassium relationships in corn leaves. Knauss also reported a linear relationship between the exchangeable potassium in soil and per cent potassium in corn leaves. Viets, et al (13) reported that the sum of cations for each treatment was nearly constant.

MATERIALS AND METHODS

Chemical Analysis of the Soil

Soil material from the experimental sites was analyzed by methods similar to ones employed in the Kansas State soil testing laboratory. The laboratory determinations included pH, lime requirement, available phosphorus, exchangeable potassium and organic matter.

The pH of the soil was determined by use of a Beckman glass electrode. Ten mls of water were added to 10 gms of soil. The mixture was stirred, allowed to stand for 15 minutes, stirred again and then the determination made. Lime requirement was made on each sample by using Woodruff's buffer (14). Twenty mls of the buffer solution were added to each of the samples

and after 30 minutes the pH was again determined. For every tenth of a pH unit under pH 7.0 one thousand pounds of lime per acre were recommended.

Available phosphorus was determined by a modification of Bray's sulfonic acid reduction colorimetric method. The extracting solution was 0.025 N HCl plus 0.03 N NH_4F. The extracting ratio of soil to solution was 1 to 50 (Smith, et al, 11). The Evelyn photoelectric colorimeter was used to measure intensity of the color produced.

In order to determine exchangeable potassium in the soils, 5 gms of soil were shaken for 10 minutes in 1.0 N $NH_4C_2H_3O_2$, pH 7.0. After filtering, 20 mls of this solution were added to 2 mls of solution containing 1100 ppm Lithium as $LiNO_3$. The resulting solution was passed through a Perkin-Elmer flame photometer and the content of potassium determined by use of a standard curve.

The organic matter of the soil was determined by Peech's titration method (9). Barium diphenylamine sulfonate was used as the indicator.

The results of the soil tests are recorded in Table 1.

Table 1. Chemical properties of soil.

Farm and Location	pH	Organic matter %	Pounds per acre		
			Lime requirement	Available phosphorus	Exchangeable potassium
Joe Campbell, Rossville	5.7	1.88	3,000	14	480
Roscoe Ellis, Sr., Havensville	5.8	3.99	2,000	125	540

Experimental Design

Two locations were used in this experiment, both in northeastern Kansas. The fertilizer treatments were different at each location. The soil test results, presented in Table 1, indicated that the Ellis farm was sufficiently high in both phosphorus and potassium to make additions of these elements unnecessary. Because of this only nitrogen treatments were employed. A randomized block design was used for this location.

The treatments used at the Ellis farm are listed in Table 2. The treatments were designed to give a comparison between three different nitrogen carriers, NH_4NO_3, $(NH_4)_2SO_4$, and NH_2CONH_2. Also, sidedressings of nitrogen were compared with applications made at planting.

Table 2. Fertilizer treatments employed at the Roscoe Ellis, Sr. farm

Treatment No.	Pounds N per acre at planting	Type of nitrogen carrier	
1	No treatment		
2	20	NH_4NO_3	
3	40	NH_4NO_3	
4	80	NH_4NO_3	
5	120	NH_4NO_3	
6	160	NH_4NO_3	
7	20	$(NH_4)_2SO_4$	
8	40	$(NH_4)_2SO_4$	
9	80	$(NH_4)_2SO_4$	
10	120	$(NH_4)_2SO_4$	
11	160	$(NH_4)_2SO_4$	
12	20	NH_2CONH_2	
13	40	NH_2CONH_2	
14	80	NH_2CONH_2	
15	120	NH_2CONH_2	
16	160	NH_2CONH_2	
17	40	NH_4NO_3	SD[1]
18	80	NH_4NO_3	SD
19	120	NH_4NO_3	SD
20	40	$(NH_4)_2SO_4$	SD
21	80	$(NH_4)_2SO_4$	SD
22	120	$(NH_4)_2SO_4$	SD
23	40	NH_2CONH_2	SD
24	80	NH_2CONH_2	SD

1 Fertilizer sidedressed

Soil test information for the Campbell farm indicated that corn at this location might respond to applications of starter fertilizers. Therefore a split plot design was used employing different starter fertilizers for main plots and rates of nitrogen as subplots. The starter fertilizers were banded in the row at planting time. The nitrogen was broadcast in the form of $(NH_4)_2SO_4$ or applied as anhydrous NH_3 just prior to planting. The treatments are listed in Table 3.

Table 3. Fertilizer treatments employed at the Joe Campbell farm.

Treatment No.	Starter fertilizer	Pounds of N per acre
1	No treatment	0
2	0	80
3	0	120*
4	0	120
5	0	160
6	15-15-0 267#/A	0
7	15-15-0 267#/A	80
8	15-15-0 267#/A	120*
9	15-15-0 267#/A	120
10	15-15-0 267#/A	160
11	12-12-12 333#/A	0
12	12-12-12 333#/A	80
13	12-12-12 333#/A	120*
14	12-12-12 333#/A	120
15	12-12-12 333#/A	160
16	10-20-0 200#/A	0
17	10-20-0 200#/A	80
18	10-20-0 200#/A	120*
19	10-20-0 200#/A	120
20	10-20-0 200#/A	160
21	10-20-10 200#/A	0
22	10-20-10 200#/A	80
23	10-20-10 200#/A	120*
24	10-20-10 200#/A	120
25	10-20-10 200#/A	160

1. All of the nitrogen except that marked (*) was applied as $(NH_4)_2SO_4$. Anhydrous NH_3 was used for treatments marked (*).

Collection and Preparation of Leaf Samples for Analysis

The third leaf from the base of the corn plants was used in this investigation. The leaf samples were gathered at varying stages of growth as follows:

Joe Campbell farm:

1. First sampling--corn approximately 1 foot tall.
2. Second sampling--corn approximately 3 feet tall.
3. Third sampling--corn tasseling.
4. Fourth sampling--corn in early ear stage, pollination completed.

Roscoe Ellis, Sr. farm:

1. First sampling--corn approximately 3 feet tall.
2. Second sampling--corn tasseling.
3. Third sampling--corn in early ear stage, pollination completed.

The sidedressed plots at the Ellis farm were sampled at the last two stages only because the sidedressing was not applied until shortly after the first sampling.

After sampling, leaf samples were dried at approximately 60 degrees centigrade for three to five days. They were then ground in a Wiley mill. The samples were dried in an oven at 100 degrees centigrade for 48 hours prior to weighing for analysis.

Chemical Analysis of Corn Leaves

The corn leaves from the Ellis farm were analyzed only for nitrogen content because nitrogen was the only fertilizer nutrient added. A one gm sample of the ground plant material was used for the determination. The

Kjeldahl-Gunning method (1) with slight modifications was used for this analysis.

The leaf samples from the Campbell farm were analyzed for nitrogen by the method previously described. Also, they were analyzed for Na, K, Ca, Mg, and P. All determinations except nitrogen, were made using a wet digestion procedure on the plant material.

The digestion procedure is essentially that prescribed by Early (6). One gm of ground plant material was placed in a 150 ml beaker. Fifteen mls of concentrated HNO_3 were mixed with the plant material and allowed to stand for 30 minutes. At the end of 30 minutes, 10 mls of water and 8 mls of concentrated $HClO_4$ were added to the mixture. The mixture was covered with a a watch glass and placed on a steam plate for three hours. After heating on the steam plate for three hours, the sides of the beaker were washed down with water, six glass beads were added, and the sample placed on an electric hot plate. The temperature of the hot plate was adjusted until the solution boiled gently. The samples were removed from the hot plate when the solution became clear and dense white fumes were evolved. The temperature of the hot plate is reduced and the samples taken to dryness.

The samples were dissolved in 25 mls of 1 N HCl. After the acid was added they were heated for 45 minutes on a steam plate, after which they were filtered into 100 ml volumetric flask and diluted to volume with distilled water. The procedure for determining each ion is given briefly in the following paragraphs.

Sodium in the plant digest was determined photometrically by use of a Beckman spectrophotometer with a flame attachment. A photomultiplier was used to increase the sensitivity of the instrument. The method of analysis

was based on suggestions given in the Beckman bulletin (2). After location of the sodium line, 291 mu on this instrument, the instrument was adjusted to 100 per cent transmittancy for a concentration range of 0-4 ppm sodium. A standard curve was made using NaCl solutions in 0.25 N HCl as standards. Calcium, Mg, and K were added to the sodium standards in the approximate concentrations that were anticipated to be in the plant digest. The unknown solution was passed through the flame and the per cent transmittancy was read and then compared to the standard curve.

Calcium in the digest was determined by a method similar to the above method for sodium. The calcium line was located at 556 mu on this instrument. After the calcium line was located the instrument was adjusted for a range of 0-100 ppm calcium. The unknown solution was passed through the flame and the per cent transmittancy read and then compared to a standard curve.

Magnesium in the plant digest was determined by passing the unknown solution through the flame photometer using the determined magnesium wavelength of 285.5 mu after the instrument had been adjusted for a concentration range of 0-40 ppm magnesium. The per cent transmittancy was read and the reading compared with a standard curve prepared by the use of standards made from magnesium ribbon dissolved in 0.25 N HCl. As was true with all standards, the magnesium standards contained amounts of other ions approximately equal to the ion content of the plant digest.

Phosphorus concentration in the digest solution was determined by the molybdenum blue method as proposed by Bray (4). A 2 ml aliquot of the digest was diluted with distilled water to a volume of 50 mls in a volumetric flask. Two mls each of $(NH_4)_2MoO_4$--HCl solution and 1-amino-2-naphthol-4-sulfonic acid in solution with Na_2SO_3 and $Na_2S_2O_5$ was added. After 15 minutes the

intensity of the color developed was measured by use of an Evelyn photoelectric colorimeter with a 660 mu filter in place.

The per cent transmittancy was determined and compared to a curve made from known concentrations of KH_2PO_4.

Potassium content in the digest was determined by use of the Beckman flame photometer. Two mls of the digest were diluted to volume in a 100 ml volumetric flask. The solution was passed through the flame after it had been adjusted to the potassium line, 771 mu and standardized with a KCl solution for a concentration range of 0-15 ppm potassium. The per cent transmittancy was read and compared with a standard curve.

DISCUSSION OF RESULTS

Nitrogen Effects

The data for nitrogen content of corn leaves are reported in Tables 4 to 8 inclusive. Figures 1 and 2 present graphically the nitrogen content of the corn leaves for certain treatments at various stages of growth.

Time of sampling is very important insofar as nitrogen content of corn leaves is concerned. Highest nitrogen concentration was found in young corn leaves. The content of nitrogen declined steadily throughout the growing season. As indicated by Fig. 1, the rate of decline is greater between the first and second samplings than between subsequent samplings.

Application of nitrogen equivalent to 80 pounds per acre on the Campbell farm resulted in highly significant increases in nitrogen content of corn leaves at each sampling. Applications of nitrogen greater than 80 pounds had little effect. At the time of the first sampling, treatments containing

starter fertilizers resulted in highly significant increases in nitrogen content of corn leaves as compared to treatments containing no starter fertilizers. However, in all subsequent samplings starter fertilizers resulted in a decrease in nitrogen content of corn leaves. In no case did use of anhydrous NH_3 result in a significant difference in nitrogen content of corn leaves as compared to equivalent rates of $(NH_4)_2SO_4$.

Additions of nitrogen fertilizer at the Ellis farm increased the nitrogen content of the corn leaves. The increase was significant for all rates of nitrogen fertilizer above 40 pounds per acre. Applications of nitrogen greater than 80 pounds per acre caused additional increases in nitrogen content of corn leaves. However, these additional increases were relatively less than those caused by application of nitrogen of less than 80 pounds per acre.

There was very little difference between nitrogen content of corn leaves from plots which received equivalent rates of nitrogen from different nitrogen carriers.

Nitrogen content of corn leaves at the time of second sampling was increased by sidedressings of nitrogen. However, nitrogen content of corn leaves from sidedressed plots was considerably lower at the time of the second sampling than was the nitrogen content of leaves from plots which received equivalent rates of nitrogen at planting. At the third sampling the nitrogen content of corn leaves from sidedressed plots was not significantly different from the nitrogen content of leaves from plots which received equivalent amounts of nitrogen at planting. This would suggest that corn leaves should be sampled late in the season if a useful measure is to be obtained. This is true particularly where corn has been sidedressed late.

It is difficult to establish a precise "critical lower limit" with respect to nitrogen contained in the leaves on the basis of one years data. However, from this years data it is suggested that the critical lower limits are as follows:

Roscoe Ellis, Sr. farm:

Corn 3 feet in height-----------------------3.00% N

Corn tasseling------------------------------2.50% N

Corn in early ear---------------------------1.90% N

Joe Campbell farm:

Corn 3 feet in height-----------------------3.15% N

Corn tasseling------------------------------2.75% N

Corn in early ear---------------------------2.25% N

Higher critical lower limits on the Campbell farm apparently prevailed because it was an irrigated field.

Cation Relationships

The data for the cation content of corn leaves are reported in Tables 9 to 16 and 21 to 28.

Calcium: Addition of nitrogen resulted in significant increases in calcium content of corn leaves in all samplings and highly significant increases in all but the first sampling. There was very little difference in calcium content of corn leaves due to different rates of nitrogen applied as $(NH_4)_2SO_4$. Use of anhydrous NH_3 as a source of nitrogen resulted in a higher average calcium content than did use of an equivalent amount of nitrogen from $(NH_4)_2SO_4$. Application of starter fertilizers gave highly significant decreases in calcium content of corn leaves at time of first sampling. However, there was little

difference in calcium content in corn leaves due to application of starter fertilizers at time of later samplings. In general, calcium content of corn leaves at the time of first sampling was lower than at later samplings.

Magnesium: Magnesium content of corn leaves was significantly increased at each sampling date by the use of nitrogenous fertilizer. In general, additions of nitrogen as anhydrous NH_3 resulted in lower magnesium content in the corn leaf than did addition of equivalent amounts of nitrogen applied as $(NH_4)_2SO_4$. This effect was highly significant at the third sampling date.

Magnesium-potassium relationships were in evidence at the time of first sampling. Application of potassium decreased the magnesium content of corn leaves. In general, the use of any starter fertilizer tended to decrease the magnesium content of corn leaves at all samplings.

Potassium: Application of starter fertilizers which contained potassium resulted in highly significant increases in potassium content of corn leaves at the time of the first three samplings. The fourth sampling reflected no significant effect. An increase in potassium content of corn leaves at time of first and third samplings was caused by starter fertilizers which contained no potassium. This possibly could be attributed to the release of some fixed potassium from clays by starter fertilizers. Further evidence of such release of potassium was furnished by treatments which included anhydrous NH_3. In every case the average potassium content of corn leaves from plots receiving anhydrous NH_3 was greater than that in leaves from plots receiving $(NH_4)_2SO_4$ in such amounts as to supply the same quantity of nitrogen. This effect was significant in the third sampling.

Sodium: Sodium content of corn leaves was so small at time of each sampling that it may be considered of little importance in the nutrition of

corn. The third sampling showed a significant increase in sodium content due to application of nitrogenous fertilizer. In the fourth sampling the sodium content of corn leaves from the 10-20-10 treatment was significantly greater than sodium content of the corn leaves from check plots.

Phosphorus Relationships

The data for the phosphorus content of corn leaves are reported in Tables 17 to 20 inclusive.

Phosphorus content of corn leaves dropped markedly between the first and second samplings. There was very little variation in phosphorus content for the last three samplings. Addition of starter fertilizers resulted in highly significant increases in phosphorus content of corn leaves in all but the second sampling.

Additions of nitrogen increased phosphorus content in the first two samplings. This increase was significant in the second sampling. There was very little difference in phosphorus content due to application of nitrogenous fertilizer at later samplings.

Table 4. Nitrogen concentration of corn leaves (Roscoe Ellis, Sr. farm).

Treatment No.	Per cent nitrogen in the corn leaves[1]		
	1st sampling	2nd sampling	3rd sampling
1	2.42	1.92	1.07
2	2.48	2.11	1.58
3	2.63	2.34	1.85
4	3.07	2.53	1.97
5	3.22	2.62	2.08
6	3.34	2.73	2.20
7	2.66	2.10	1.47
8	2.77	2.29	1.70
9	2.92	2.51	1.88
10	3.06	2.62	2.16

Table 4 (cont.)

Treatment No.	Per cent nitrogen in the corn leaves[1] 1st sampling	2nd sampling	3rd sampling
11	3.28	2.55	2.01
12	2.60	2.15	1.63
13	2.72	2.25	1.69
14	3.06	2.51	1.95
15	3.05	2.52	2.05
16	3.29	2.68	2.14
17		2.02	1.72
18		2.26	1.88
19		2.16	1.89
20		1.98	1.69
21		2.09	1.83
22		2.08	1.83
23		2.24	1.78
24		2.25	1.88
L.S.D. (.05)	.23	.20	.21
(.01)	.32	.27	.28

Table 5. Nitrogen concentration of corn leaves, first sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Nitrogen content of corn leaves (per cent)[1] 0N	80N	120N	120N[2]	160N	Average
None	3.77	3.93	4.02	4.08	4.34	4.03
15-15-0	4.18	4.29	4.38	4.34	4.42	4.32
12-12-12	3.96	4.17	4.26	4.25	4.46	4.22
10-20-0	3.74	4.08	4.37	4.25	4.39	4.17
10-20-10	3.76	3.98	4.20	4.12	3.97	4.01
Average	3.88	4.09	4.25	4.21	4.32	

L.S.D. for nitrogen treatments (.05) .15
(.01) .20
L.S.D. for starter fertilizers (.05) .14
(.01) .19

1 Each value reported is an average of four replications.
2 Applied as anhydrous NH_3.

Table 6. Nitrogen concentration of corn leaves, second sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Nitrogen content of corn leaves (per cent)[1] 0N	80N	120N	120N[2]	160N	Average
None	2.67	3.16	3.19	3.32	3.27	3.12
15-15-0	2.45	3.08	3.12	3.03	3.11	2.96
12-12-12	2.41	2.99	3.02	3.03	2.97	2.88
10-20-0	2.42	2.95	3.24	2.96	3.01	2.92
10-20-10	2.65	2.95	3.08	2.99	2.96	2.93
Average	2.52	3.03	3.13	3.07	3.06	

L.S.D. for nitrogen treatments (.05) .13
(.01) .17
L.S.D. for starter fertilizers (.05) .06
(.01) .08

1 Each value reported is an average of four replications
2 Applied as anhydrous NH_3.

Table 7. Nitrogen concentration of corn leaves, third sampling (Joe Campbell farm)

Starter Fertilizer Treatment	Nitrogen content of corn leaves (per cent)[1] 0N	80N	120N	120N[2]	160N	Average
None	2.34	2.75	2.73	2.64	2.84	2.66
15-15-0	2.13	2.58	2.70	2.65	2.76	2.56
12-12-12	2.13	2.65	2.68	2.48	2.66	2.52
10-20-0	1.99	2.80	2.63	2.59	2.64	2.53
10-20-10	2.12	2.52	2.66	2.72	2.53	2.51
Average	2.14	2.66	2.68	2.62	2.69	

L.S.D. for nitrogen treatments (.05) .12
(.01) .17
L.S.D. for starter fertilizers (.05) .08
(.01) .11

1 Each value reported is an average of four replications.
2 Applied as anhydrous NH_3.

Table 8. Nitrogen concentration of corn leaves, fourth sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Nitrogen content of corn leaves (per cent)[1] ON	80N	120N	120N[2]	160N	Average
None	1.77	2.23	2.17	2.19	2.28	2.13
15-15-0	1.79	2.16	2.22	2.15	2.17	2.10
12-12-12	1.74	2.16	2.27	2.10	2.12	2.08
10-20-0	1.88	2.15	2.16	2.06	2.17	2.08
10-20-10	1.68	2.16	2.21	2.12	2.22	2.08
Average	1.77	2.17	2.21	2.12	2.19	

L.S.D. for nitrogen treatments (.05) .09
(.01) .12

Table 9. Calcium concentration of corn leaves, first sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Calcium content of corn leaves (per cent)[1] ON	80N	120N	120N[2]	160N	Average
None	.182	.270	.218	.222	.229	.224
15-15-0	.142	.172	.164	.166	.157	.164
12-12-12	.130	.145	.132	.148	.146	.140
10-20-0	.136	.149	.142	.138	.136	.140
10-20-10	.121	.132	.126	.143	.124	.129
Average	.142	.174	.156	.167	.158	

L.S.D. for nitrogen treatments (.05) .019

L.S.D. for starter fertilizers (.05) .028
(.01) .039

1 Each value reported is an average of four replications.
2 Applied as anhydrous NH_3.

Table 10. Calcium concentration of corn leaves, second sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Calcium content of corn leaves (per cent)[1] 0N	80N	120N	120N[2]	160N	Average
None	.336	.386	.344	.399	.337	.360
15-15-0	.354	.409	.439	.462	.434	.420
12-12-12	.388	.406	.420	.467	.399	.416
10-20-0	.303	.448	.458	.451	.405	.413
10-20-10	.496	.462	.461	.449	.434	.460
Average	.375	.422	.424	.446	.402	
L.S.D. for nitrogen treatments (.05)			.040			

Table 11. Calcium concentration of corn leaves, third sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Calcium content of corn leaves (per cent)[1] 0N	80N	120N	120N[2]	160N	Average
None	.320	.370	.317	.429	.323	.352
15-15-0	.323	.503	.446	.470	.422	.433
12-12-12	.387	.472	.445	.410	.389	.421
10-20-0	.354	.475	.408	.403	.413	.411
10-20-10	.346	.400	.511	.469	.398	.425
Average	.346	.444	.425	.436	.389	
L.S.D. for nitrogen treatments (.05)			.039			
(.01)			.052			
L.S.D. for starter fertilizers (.05)			.055			

1 Each value reported is an average of four replications.
2 Applied as anhydrous NH_3.

Table 12. Calcium concentration of corn leaves, fourth sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Calcium content of corn leaves (per cent)[1]					Average
	0N	80N	120N	120N[2]	160N	
None	.369	.389	.349	.457	.354	.384
15-15-0	.343	.414	.404	.432	.398	.398
12-12-12	.374	.393	.395	.434	.406	.400
10-20-0	.318	.429	.426	.438	.422	.407
10-20-10	.371	.389	.384	.454	.392	.398
Average	.355	.403	.392	.443	.394	

L.S.D. for nitrogen treatments (.05) .029
(.01) .039

Table 13. Magnesium concentration of corn leaves, first sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Magnesium content of corn leaves (per cent)[1]					Average
	0N	80N	120N	120N[2]	160N	
None	.214	.273	.260	.235	.251	.247
15-15-0	.268	.278	.253	.290	.260	.270
12-12-12	.193	.195	.212	.192	.202	.199
10-20-0	.201	.234	.208	.169	.316	.226
10-20-10	.188	.228	.199	.158	.144	.183
Average	.213	.242	.226	.209	.235	

L. S. D. for nitrogen treatments (.05) .022
L. S. D. for starter fertilizers (.05) .019
(.01) .027

1 Each value reported is an average of four replications.

2 Applied as anhydrous NH_3.

Table 14. Magnesium concentration of corn leaves, second sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Magnesium content of corn leaves (per cent)[1] 0N	80N	120N	120N[2]	160N	Average
None	.209	.312	.276	.242	.232	.254
15-15-0	.182	.245	.208	.203	.174	.202
12-12-12	.150	.167	.269	.329	.308	.245
10-20-0	.234	.334	.308	.325	.288	.298
10-20-10	.268	.285	.305	.288	.262	.282
Average	.209	.269	.273	.277	.253	

L.S.D. for nitrogen treatments (.05) .030
(.01) .040
L.S.D. for starter fertilizers (.05) .040
(.01) .056

Table 15. Magnesium concentration of corn leaves, third sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Magnesium content of corn leaves (per cent)[1] 0N	80N	120N	120N[2]	160N	Average
None	.258	.338	.320	.312	.279	.301
15-15-0	.222	.338	.335	.292	.328	.303
12-12-12	.261	.337	.298	.208	.222	.265
10-20-0	.170	.276	.328	.236	.284	.259
10-20-10	.189	.228	.277	.228	.284	.241
Average	.220	.303	.312	.255	.279	

L.S.D. for nitrogen treatments (.05) .033
(.01) .043
L.S.D. for starter fertilizers (.05) .029
(.01) .041

1 Each value reported is an average of four replications.
2 Applied as anhydrous NH_3.

Table 16. Magnesium concentration of corn leaves, fourth sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Magnesium content of corn leaves (per cent)[1]					Average
	0N	80N	120N	120N[2]	160N	
None	.233	.311	.261	.260	.254	.264
15-15-0	.211	.269	.256	.267	.242	.249
12-12-12	.239	.223	.258	.256	.273	.250
10-20-0	.184	.296	.315	.309	.305	.282
10-20-10	.210	.246	.286	.208	.172	.224
Average	.215	.269	.275	.260	.249	

L.S.D. for nitrogen treatments (.05) .029
(.01) .038
L.S.D. for starter fertilizers (.05) .018
(.01) .025

Table 17. Phosphorus concentration of corn leaves, first sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Phosphorus content of corn leaves (per cent)[1]					Average
	0N	80N	120N	120N[2]	160N	
None	.318	.211	.259	.244	.239	.254
15-15-0	.461	.537	.507	.476	.507	.498
12-12-12	.456	.462	.480	.548	.627	.515
10-20-0	.511	.510	.569	.484	.567	.528
10-20-10	.521	.586	.562	.546	.568	.557
Average	.453	.461	.475	.460	.502	

Significant interaction between effects of nitrogen and starter fertilizers.
L.S.D. for starter fertilizers (.05) .101
(.01) .142

1 Each value reported is an average of four replications.
2 Applied as anhydrous NH_3.

Table 18. Phosphorus concentration of corn leaves, second sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Phosphorus content of corn leaves (per cent)[1]					Average
	0N	80N	120N	120N[2]	160N	
None	.220	.191	.202	.215	.218	.209
15-15-0	.210	.255	.241	.225	.233	.233
12-12-12	.212	.252	.224	.234	.214	.227
10-20-0	.207	.258	.258	.225	.220	.234
10-20-10	.235	.259	.255	.254	.220	.245
Average	.217	.243	.236	.231	.221	

L.S.D. for nitrogen treatments (.05) .020

Table 19. Phosphorus concentration of corn leaves, third sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Phosphorus content of corn leaves (per cent)[1]					Average
	0N	80N	120N	120N[2]	160N	
None	.209	.181	.188	.202	.219	.200
15-15-0	.213	.207	.232	.219	.218	.218
12-12-12	.201	.205	.208	.216	.226	.211
10-20-0	.232	.244	.233	.234	.233	.235
10-20-10	.232	.226	.215	.242	.230	.229
Average	.217	.213	.215	.223	.225	

Significant interaction between effects of nitrogen and starter fertilizers.
L.S.D. for starter fertilizers (.05) .016
(.01) .023

1 Each value reported is an average of four replications.
2 Applied as anhydrous NH_3.

Table 20. Phosphorus concentration of corn leaves, fourth sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Phosphorus content of corn leaves (per cent)[1]					Average
	0N	80N	120N	120N[2]	160N	
None	.207	.198	.200	.202	.202	.202
15-15-0	.234	.213	.230	.219	.222	.224
12-12-12	.217	.209	.210	.190	.192	.204
10-20-0	.214	.204	.210	.200	.212	.208
10-20-10	.224	.219	.214	.209	.214	.216
Average	.219	.209	.213	.204	.208	

L.S.D. for starter fertilizers (.05) .012
(.01) .016

Table 21. Potassium concentration of corn leaves, first sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Potassium content of corn leaves (per cent)[1]					Average
	0N	80N	120N	120N[2]	160N	
None	4.62	4.29	4.59	4.58	4.74	4.56
15-15-0	4.51	5.29	4.95	5.17	5.01	4.99
12-12-12	5.16	5.39	4.75	5.78	5.54	5.32
10-20-0	4.25	4.76	5.65	5.22	5.81	5.14
10-20-10	5.21	6.22	5.71	5.64	6.20	5.80
Average	4.75	5.19	5.13	5.28	5.46	

L.S.D. for starter fertilizers (.05) .45
(.01) .63

1 Each value reported is an average of four replications.
2 Applied as anhydrous NH_3.

Table 22. Potassium concentration of corn leaves, second sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Potassium content of corn leaves (per cent)[1]					Average
	0N	80N	120N	120N[2]	160N	
None	3.44	3.59	3.55	3.74	3.45	3.55
15-15-0	3.78	3.25	3.18	3.50	3.32	3.40
12-12-12	3.59	3.44	3.65	3.81	3.86	3.67
10-20-0	3.77	3.69	3.33	3.79	3.70	3.66
10-20-10	3.82	3.90	3.86	4.14	4.06	3.96
Average	3.68	3.57	3.51	3.80	3.68	

L.S.D. for starter fertilizers (.05) .28
(.01) .40

Table 23. Potassium concentration of corn leaves, third sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Potassium content of corn leaves (per cent)[1]					Average
	0N	80N	120N	120N[2]	160N	
None	2.04	1.91	2.01	2.56	2.40	2.18
15-15-0	2.96	2.61	2.38	2.78	2.60	2.67
12-12-12	2.90	2.46	2.82	3.19	3.24	2.92
10-20-0	2.94	2.52	2.58	2.64	2.44	2.62
10-20-10	2.98	2.80	2.93	2.99	2.84	2.91
Average	2.76	2.46	2.54	2.83	2.70	

L.S.D. for nitrogen treatments (.05) .21
(.01) .27
L.S.D. for starter fertilizers (.05) .29
(.01) .41

1 Each value reported is an average of four replications.
2 Applied as anhydrous NH_3.

Table 24. Potassium concentration of corn leaves, fourth sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Potassium content of corn leaves (per cent)[1]					Average
	0N	80N	120N	120N[2]	160N	
None	2.54	2.14	2.48	2.62	2.38	2.43
15-15-0	2.59	1.91	2.18	2.37	2.35	2.28
12-12-12	2.51	2.56	2.30	2.42	2.34	2.43
10-20-0	2.46	2.20	2.52	2.48	2.48	2.43
10-20-10	2.76	2.55	2.59	2.41	2.60	2.58
Average	2.57	2.27	2.41	2.46	2.43	

No significant differences

Table 25. Sodium concentration of corn leaves, first sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Sodium content of corn leaves (per cent)[1]					Average
	0N	80N	120N	120N[2]	160N	
None	.007	.010	.007	.008	.007	.008
15-15-0	.005	.008	.009	.006	.005	.007
12-12-12	.008	.006	.005	.008	.007	.007
10-20-0	.006	.007	.006	.006	.006	.006
10-20-10	.007	.009	.010	.007	.007	.008
Average	.007	.008	.007	.007	.006	

No significant differences

1 Each value reported is an average of four replications.
2 Applied as anhydrous NH_3.

Table 26. Sodium concentration of corn leaves, second sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Sodium content of corn leaves (per cent)[1]					Average
	0N	80N	120N	120N[2]	160N	
None	.004	.002	.004	.004	.003	.003
15-15-0	.002	.002	.004	.004	.004	.003
12-12-12	.004	.005	.004	.004	.004	.004
10-20-0	.003	.003	.004	.004	.003	.003
10-20-10	.003	.004	.003	.002	.004	.003
Average	.003	.003	.004	.004	.004	

Significant interaction between effects of nitrogen and starter fertilizers.

Table 27. Sodium concentration of corn leaves, third sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Sodium content of corn leaves (per cent)[1]					Average
	0N	80N	120N	120N[2]	160N	
None	.001	.002	.001	.001	.002	.001
15-15-0	.001	.002	.002	.002	.002	.002
12-12-12	.002	.001	.003	.006	.007	.004
10-20-0	.004	.007	.006	.006	.006	.006
10-20-10	.003	.005	.006	.006	.006	.005
Average	.002	.003	.004	.004	.005	

L.S.D. for nitrogen treatments (.05) .001
(.01) .002

1 Each value reported is an average of four replications.
2 Applied as anhydrous NH_3.

Table 28. Sodium concentration of corn leaves, fourth sampling (Joe Campbell farm).

Starter Fertilizer Treatment	Sodium content of corn leaves (per cent)[1]					Average
	0N	80N	120N	120N[2]	160N	
None	.005	.001	.005	.003	.002	.003
15-15-0	.002	.003	.002	.004	.002	.003
12-12-12	.001	.003	.003	.003	.004	.003
10-20-0	.004	.004	.006	.005	.004	.005
10-20-10	.006	.006	.006	.004	.006	.006
Average	.004	.003	.004	.004	.004	

L.S.D. for starter fertilizers (.05) .002

1 Each value reported is an average of four replications.
2 Applied as anhydrous NH_3.

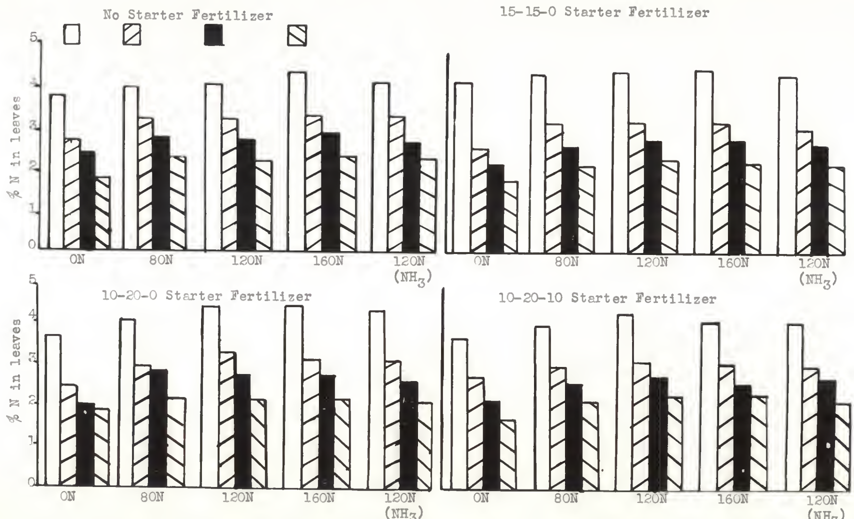

Fig. 1. Effect of fertilizer application on nitrogen content of corn leaves at Joe Campbell farm - Rossville, Kansas, 1954.

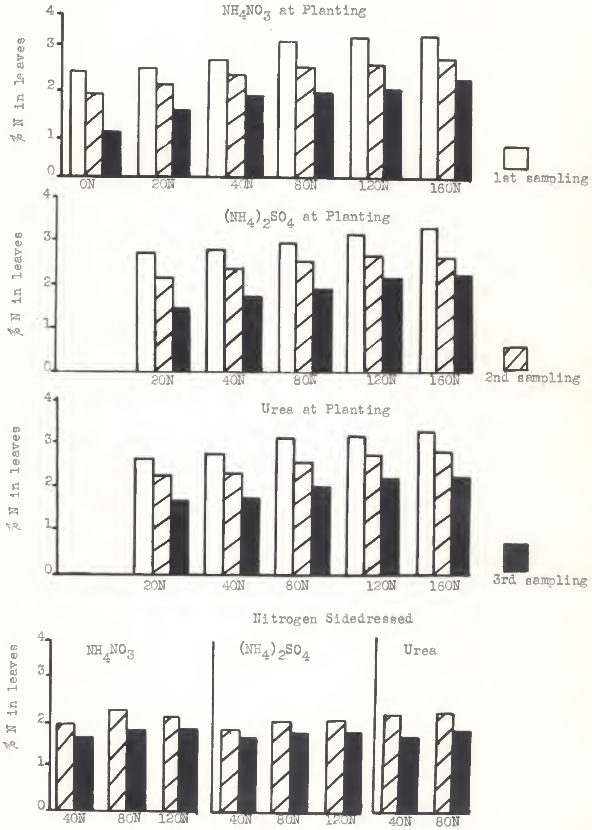

Fig. 2. Effect of fertilizer application on nitrogen content of corn leaves at Roscoe Ellis farm - Havensville, Kansas - 1954.

SUMMARY

An investigation was conducted in conjunction with two 1954 corn fertilizer experiments, one an irrigated field and the other a non-irrigated field, to study nitrogen nutrition of corn by use of leaf analysis. Also, various cation relationships were studied on the corn leaves from the irrigated experiment.

Leaf samples were collected at various stages of growth and analyzed in the laboratory.

From an examination of the results the following conclusions were drawn:

1. Time of sampling is very important insofar as nutrient content of the corn plant is concerned. The various elements tend to change as the growing season progresses as follows:

   a. Nitrogen content decreases.

   b. Calcium content increases.

   c. Magnesium content increases.

   d. Sodium content remains fairly constant.

   e. Potassium content decreases.

   f. Phosphorus content decreases.

2. Applications of nitrogenous fertilizers increases the per cent nitrogen in the corn leaf.

3. Nitrogen content of the corn leaf tends to remain constant for equivalent rates of nitrogen applied regardless of the type of fertilizer furnishing the nitrogen.

4. Corn leaves should be sampled late if a useful measure of nitrogen content of the leaves is to be obtained. This is particularly true where corn has been sidedressed late.

5. Critical lower limits of nitrogen are suggested as follows:

Roscoe Ellis, Sr. farm:

Corn 3 feet in height------------------------3.00% N

Corn tasseling-------------------------------2.50% N

Corn in early ear----------------------------1.90% N

Joe Campbell farm:

Corn 3 feet in height------------------------3.15% N

Corn tasseling-------------------------------2.75% N

Corn in early ear----------------------------2.25% N

6. Additions of nitrogen increase the calcium content of corn leaves. Use of anhydrous NH_3 as a source of nitrogen resulted in a higher average calcium content than did use of an equivalent amount of nitrogen form $(NH_4)_2SO_4$.

7. There exists a magnesium-potassium relationship. Additions of starter fertilizers containing potassium decreased the magnesium content of corn leaves.

8. Additions of nitrogen as anhydrous NH_3 lowered the magnesium content of corn leaves as compared to equivalent amounts of nitrogen added as $(NH_4)_2SO_4$.

9. Sodium content of corn leaves may be considered of little importance in the nutrition of corn.

10. Potassium content of corn leaves was increased by additions of starter fertilizers and anhydrous NH_3. This is attributed to the release of fixed potassium from clays.

11. Additions of starter fertilizers increased the phosphorus content of the corn leaves.

12. A relationship exists between nitrogen and phosphorus. Additions of nitrogen increased phosphorus content of the corn leaf.

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I INTERFERENCE OF FLUORIDE WITH COLORIMETRIC MEASUREMENT OF PHOSPHORUS

II NITROGEN NUTRITION STUDIES WITH CORN

by

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1955

PART I: INTERFERENCE OF FLUORIDE WITH COLORIMETRIC MEASUREMENT OF PHOSPHORUS

This experiment was designed to determine the effect of different concentrations of fluoride on color development in colorimetric measurement of phosphorus, and to determine to what extent the H_3BO_3 added to $(NH_4)_2MoO_4$--HCl solution may be depended upon to overcome this interference.

Standard solutions containing 0 to 4 ppm of phosphorus were prepared and phosphorus content was measured colorimetrically. This was done first in distilled water and then in aqueous solutions containing NH_4F in concentrations of 0.03 N, 0.06 N, and 0.12 N or until such concentration of fluoride as was being used resulted in total repression of color development. Phosphorus standards then were prepared similarly in 0.025 N HCl and 0.1 N HCl instead of water and similar colorimetric measurements were repeated. The above determinations were repeated using H_3BO_3 in the $(NH_4)_2MoO_4$--HCl solution.

The following conclusions were drawn:

1. Ammonium fluoride represses color development even when present in small concentrations (0.03 N).

2. Ammonium fluoride represses color development more as acidity increases.

3. Color repression is greater for greater concentrations of phosphorus.

4. Boric acid used with $(NH_4)_2MoO_4$--HCl solution is effective within the following limitations:

   a. up to 0.05 N NH_4F in water.

   b. up to 0.04 N NH_4F in 0.025 N HCl.

   c. it was not effective as low as 0.03 N NH_4F in 0.1 N HCl.

PART II: NITROGEN NUTRITION STUDIES WITH CORN

A laboratory experiment was conducted in conjunction with two 1954 corn fertilizer experiments with the following objectives in view:

1. To determine the effect of different times of sampling upon the percentage of nitrogen contained in corn leaves.

2. To determine the influence of various applications of fertilizer upon the percentage nitrogen contained in corn leaves.

3. To establish "critical levels of nitrogen" in corn leaves.

4. To determine the percentage of P, K, Ca, Na, and Mg contained in corn leaves of plots which received starter fertilizer.

A leaf sample consisting of the third leaf from the bottom of the corn plant was taken from each of the plots to be studied. The plots were sampled at various times as follows:

Joe Campbell farm:

1. First sampling---corn approximately 1 foot tall.

2. Second sampling---corn approximately 3 feet tall.

3. Third sampling---corn tasseling.

4. Fourth sampling---corn in early ear stage, pollination completed.

Roscoe Ellis, Sr. farm:

1. First sampling---corn approximately 3 feet tall.

2. Second sampling---corn tasseling.

3. Third sampling---corn in early ear stage, pollination completed.

Nitrogen was determined by a modified Kjeldahl procedure. A wet digestion procedure was used for phosphorus and cation determinations. Phosphorus was determined by the colorimetric molybdenum blue procedure. Sodium, Ca, K, and Mg were determined by use of a Beckman spectrophotometer with a flame attachment.

The following conclusions were drawn from the experimental data:

1. Time of sampling is very important insofar as nutrient content of the corn plant is concerned. The various elements tend to change as the growing season progresses as follows:

a. Nitrogen, phosphorus, and potassium content decrease.

b. Calcium and magnesium content increase.

c. Sodium content remains fairly constant.

2. Applications of nitrogenous fertilizers increases the per cent nitrogen in the corn leaf.

3. Nitrogen content of the corn leaf tends to remain constant for equivalent rates of nitrogen applied regardless of the type of fertilizer furnishing the nitrogen.

4. Corn leaves should be sampled late if a useful measure of nitrogen content of the leaves is to be obtained. This is particularly true where corn has been sidedressed late.

5. Critical lower limits of nitrogen are suggested as follows:

Roscoe Ellis, Sr. farm:

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Corn tasseling--------------------------2.75% N

Corn in early ear-----------------------2.25% N

6. Additions of nitrogen increase the calcium content of corn leaves. Use of anhydrous NH_3 as a source of nitrogen resulted in a higher average calcium content than did use of an equivalent amount of nitrogen from $(NH_4)_2SO_4$.

7. There existed a magnesium-potassium relationship. Additions of starter fertilizers containing potassium decreased the magnesium content of corn leaves.

8. Additions of nitrogen as anhydrous NH_3 lowered the magnesium content of corn leaves as compared to equivalent amounts of nitrogen added as $(NH_4)_2SO_4$.

9. Sodium content of corn leaves may be considered of little importance in the nutrition of corn.

10. Potassium content of corn leaves was increased by additons of starter fertilizers and anhydrous NH_3. This is attributed to the release of fixed potassium from clays.

11. Additions of starter fertilizers increased the phosphorus content of the corn leaves.

12. A relationship existed between nitrogen and phosphorus. Additions of nitrogen increased phosphorus content of the corn leaf.